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(54) Title: REDUCING LIKELIHOOD OF VASCULAR DISORDERS IN SUSCEPTIBLE PATIENTS

(57) Abstract

A method of reducing the likelihood of heart attacks, strokes or peripheral vascular diseases in a patient susceptible thereto comprising administering to such patient an amount effective therefor of vitamin B_6 plus at least one of betaine, choline and lecithin. In addition there may be administered at least one of folic acid and vitamin B_{12}.

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REDUCING LIKELIHOOD OF VASCULAR DISORDERS IN SUSCEPTIBLE PATIENTS

The present invention relates to reducing the likelihood of vascular disorders in susceptible patients.

BACKGROUND OF THE INVENTION

It is known that elevated levels of plasma or serum homocysteine, a condition called hyperhomocysteinemia, evidence the possibility of premature vascular disease of the heart (coronary artery disease), of the brain (cerebrovascular disease), and of the periphery (peripheral vascular disease).

The fact that plasma or serum levels of homocysteine are elevated in the foregoing conditions is shown in the following references:

1. CORONARY ARTERY DISEASE
Kang et al. J Clin Invest 77: 1482-1486, 1986.
Israelson et al. Atherosclerosis 71: 227-233, 1988.
Genest et al. J Am Coll Cardiol 16: 1114-1119, 1990.
Malinow et al. Cor Art Dis 1: 215-220, 1990.
Clarke et al. New Engl J Med 324: 1149-1155, 1991.
Ubbink et al. Klin Wochenschr 69: 527-534, 1991.

2. CEREBROVASCULAR DISEASE
Clarke et al. New Engl J Med 324: 1149-1155, 1991.
Brattstrom et al. Stroke 15: 1012-1016, 1984.
Boers et al. New Engl J Med 313: 709-715, 1985.
Araki et al. Atherosclerosis 79: 139-146, 1989.
Goull et al. Stroke 21: 572-576, 1990.

3. PERIPHERAL VASCULAR DISEASE
Clarke et al. New Engl J Med 324: 1149-1155, 1991.
Malinow et al. Circulation 79: 1180-1188, 1989.
Taylor et al. J Vascul Surg 13: 128-136, 1991.
Rubba et al. Metabolism 39: 1191-1195, 1990.

HYPERHOMOCYSTEINEMIA IN PATIENTS WITH CYSTATHIONINE β-SYNTHASE DEFICIENCY

The fact that hyperhomocysteinemia often accompanies vascular disease has been reported in studies of patients with cystathionine -β-Synthase (CBS) deficiency. Vascular disease is widespread among patients with CBS deficiency (Gibson et al. J Clin Path 17: 427-437, 1964; McCully. Am J Path 56: 111-128, 1969; McCully. Atherosclerosis Rev 11: 157-246, 1983). CBS deficiency is inherited as an autosomal recessive trait. It is characterized by high levels of homocysteine, homocystine (the dimer of homocysteine), methionine and homocysteine-cysteine mixed disulfides in the plasma and urine. The common clinical features of CBS deficiency include widespread vascular disease. Severe carotid or coronary artery disease and thromboembolic pulmonary disease are causes of early death in patients with CBS deficiency. The homozygous form of the disease (recessive trait from each parent) is rare. The heterozygous form of the disease has been estimated to have a prevalence of 1 in 70 to 1 in 200 in the general population. However, hyperhomocysteinemia has been found to be prevalent in the general population without the concurrent homozygosity or heterozygosity for CBS deficiency.

HYPERHOMOCYSTEINEMIA WITHOUT CBS ENZYME DEFICIENCY IS A RISK FACTOR FOR VASCULAR DISEASE

Evidence exists that hyperhomocysteinemia accompanies vascular disease in the general population without CBS deficiency. (Genest et al. J Am Coll Cardiol 16: 1114-1119, 1990). What is amazing is that hyperhomocysteinemia may be even more common than hypercholesterolemia (Ubbink et al. Klin Wochenschr 69: 527-534, 1991). Furthermore, patients with hyperhomocysteinemia are more at risk of clinical progression of coronary and peripheral vascular disease (Taylor et al. J Vasc Surg 13: 128-136, 1991). It is estimated that between 20% and 40% in various populations with coronary heart disease have elevated levels of plasma or serum homocysteine (Malinow et al. Cor Art Dis 1: 215-220, 1990; Clarke et al. New Engl J Med 324: 1149-1155, 1991; Ubbink et al. Klin Wochenschr 69: 527-534, 1991; Ubbink et al. Am J Clin Nutr 57: 47-53, 1993).

There is experimental evidence that homocysteine accumulation in pigs induced by diets deficient in vitamin B_6 results in vascular (arterial) damage and atherosclerosis (Smolin et al. J Nutr 113:2122-2133, 1983). Moreover, there is evidence that homocysteine is injurious to endothelial cells in culture (Stamler et al. J Clin Invest 91: 308-318, 1993). In this study prolonged exposure (over 3 hours) of

ndothelial cells to homocystein resulted in impaired responses of the endothelium-derived relaxing factor (EDRF). Homocysteine support d H_2O_2 products generation and underwentconversion to homocysteine-thiolactone, products believed to contribute to endothelial toxicity. Direct chemical injury to human endothelial cells in vitro was demonstrated to be mediated through the sulphydryl group of homocysteine. Other sulphydryl compounds such as homocystine and methionine did not induce endothelial cell injury (Wall et al. Thrombosis Res 18: 113-121, 1980). In another study (Starkebaum and Harlan. J Clin Invest 77: 1370-1376, 1986) of human umbilical vein and bovine aortic endothelial cells in tissue culture, homocysteine induced endothelial cell injury that was ascribed to copper-induced hydrogen peroxide generation from homocysteine.

It is an object of the invention to reduce the likelihood of vascular disorders in susceptible patients.

This is realized in accordance with the present invention by determining in known manner those individuals who are susceptible thereto by determining the homocysteine level in their blood. Those exhibiting a homocysteine level above about 10, preferably above about 14, and especially

above about 16 micromoles/liter, are susceptible to the indicated vascular disorders.

In accordance with the invention, to such patients there are administered

i) vitamin B_6 (pyridoxine or a salt thereof such as the hydrochloride or phosphate), and

ii) at least one of
   a) betaine,
   b) choline,
   c) lecithin,
   c) vitamin B_{12} or
   d) folic acid.

This serves to reduce the level of homocysteine in the blood which, in turn, reduces the likelihood of the onset of such vascular disorders. In other words, the homocysteine level is not simply an effect or symptom of a vascular disorder but, to some extent and in a not fully understood way, is a cause of such disorders at least in part. By reducing the homocysteine level, the likelihood of onset of such disorders is reduced.

The choline can be in the form of choline per se or as a salt or ester derivative thereof such as choline chloride, choline phosphate, phosphatidyl choline, choline dihydrogen citrate, and the like. The betaine can be in free base anhydrous form or in salt or ester form such as betaine hydrochloride.

While not wishing to be bound thereby, the mechanism of the present invention is described hereinbelow in conjunction with the appended drawing wherein the sole figure is a biological flow sheet of a method for reducing the homocysteine level in the blood of a patient.

Referring now more particularly to the drawing, humans derive the amino acid methionine from the diet, but not homocysteine. Homocysteine is synthesized in the body from methionine. The steps involved in the conversion of methionine to homocysteine are presented in the Figure. In order to keep the levels of homocysteine low, the cell converts homocysteine to methionine through the process of active methylation which requires the enzyme homocysteine methyl transferase. The methyl group needed for this conversion is derived from N^5methyl tetrahydrofolate (derived from the vitamin folic acid), a process in which vitamin B_{12} plays a co-factor role. Thus, both folic acid and vitamin B_{12}

are needed for the conversion of the toxic homocystein to the non-toxic methionine. Betaine (an oxidation product of choline) also can act at the methyl donor for th conversion of homocysteine to methionine, catalyzed by the enzyme betaine-homocysteine methyl transferase.

Another mechanism exists for keeping homocysteine levels low and that is through the conversion of homocysteine to the nontoxic amino acid cysteine (see the Figure). Homocysteine combines with the amino acid serine, a reaction catalyzed by the enzyme cystathionine ß-synthase (CBS). A cofactor for this enzyme is vitamin B_6. The resultant compound, cystathionine, undergoes hydrolysis catalyzed by the enzyme cystathionase, which also requires vitamin B_6 as a co-factor. The end products are cysteine and α-ketobutyrate.

Thus, the conversion of homocysteine to methionine requires methylation via folic acid and vitamin B_{12} or a methyl donor such as betaine, choline or phosphatidyl choline (lecithin). Furthermore, the conversion of homocysteine to cysteine requires vitamin B_6.

Folic acid deficiency is associated with elevated plasma levels of homocysteine (Kang et al. Metabolism 36:

458-462, 1987; Brattstrom et al. Scand J Clin Lab Invest 48: 215-221, 1988; Wilcken et al. Metabolism 37: 697-701, 1988; Stabler et al. J Clin Invest 81: 466-477, 1988). Treatment with folic acid lowers the plasma concentration of homocysteine.

Vitamin B_6 deficiency also results in elevated plasma levels of homocysteine (Smolin et al. J Nutr 113: 2122-2133, 1983). Treatment with vitamin B_6 results in a fall in the levels of plasma homocysteine.

Vitamin B_{12} deficiency induces enormous elevations in the plasma levels of homocysteine. Treatment with vitamin B_{12} results in the normalization of the plasma levels of homocysteine (Stabler et al. J Clin Invest 81: 466-477, 1988). In a study by Brattstrom et al (Metabolism 37: 175-178, 1988), higher plasma levels of homocysteine were found in vitamin B_{12} deficiency than in heterozygosity for homocystinuria due to cystathionine ß-synthase (CBS) deficiency.

Betaine deficiency has not been described in humans who are able to synthesize betaine via the oxidation of choline or substances containing choline, such as phosphatidyl choline (lecithin). Oral administration of

betaine has been reported to lower plasma homocystine in patients with CBS deficiency (Smolin et al. J. Pediatrics 99:467-472, 1981), suggesting that circumvention of the CBS pathway is effective in lowering plasma homocysteine and therefore its dimer homocystine.

CONTROL OF HYPERHOMOCYSTEINEMIA IN HUMANS

The normal concentration of homocysteine in the plasma of humans ranges between less than 5 to 14 micromoles per liter. The treatment of hyperhomocysteinemia according to this invention involves the administration of suitable relative amounts of the specific components,

vitamin B_6 - 10 to 1000 mg, preferably 25 to 500, most preferably 50 to 100 mg;

betaine, choline and/or lecithin - 5 to 1000 mg, preferably about 25 to 750, most preferably about 50 to 500 mg;

folic acid - 0.03 to 1 mg, preferably 0.2 to 1.0 mg; and/or

B_{12} - 0.0005 to 1 mg, preferably 0.1 to 0.5 mg.

Since the components other than vitamin B_6 are alternates for one another, corresponding adjustments can be made.

The amounts indicated are for a single daily dose. If desired, the ingredients could be administered separately or smaller doses could be administered several times a day or larger doses less frequently, or in controlled release form.

The materials can be administered singly or in combination, as solids or solutions. They can be administered as tablets, capsules or ampoules or in injectable form.

The active ingredients may be administered in about 100% concentration or they may be diluted or dissolved with solids and/or liquids possibly exerting adjuvant activities, fillers, colorants, stabilizers, and the like, e.g. lactose, cellulose, ethylene glycol, propylene glycol, ascorbate, water, and the like.

Preferred compositions for daily administration in the form of a tablet to a patient whose blood exhibits a homocysteine level above about 14 micromoles/liter and especially if above about 16 micromoles/liter, are shown in the following examples wherein all parts are by weight unless

otherwise expressed. In these examples the compositions can be compacted in conventional manner to form a tablet or can be formed into a soft gelatin capsule, i.e. enclosed in a soluble film:

Example 1

Vitamin B_6 (Pyridoxine hydrochloride)	100 mg
Betaine (hydrochloride)	500 mg
Cellulose	100 mg
Butylated hydroxy anisole (BHA)	2 mg
Butylated hydroxy toluene (BHT	2 mg
Sodium Ascorbate	10 mg
Compressed tablet or soft gel capsule	

Example 2

Vitamin B6	100 mg
Choline chloride	500 mg
Cellulose	100 mg
Butylated hydroxy anisole (BHA)	2 mg
Butylated hydroxy toluene (BHT)	2 mg
Sodium Ascorbate	10 mg
Compressed tablet or soft gel capsule	

Example 3

Vitamin B_6	100 mg
Vitamin B_{12}	0.1 mg
Folic acid	0.6 mg
Cellulose	400 mg
Butylated hydroxy anisole (BHA)	2 mg
Butylated hydroxy toluene (BHT)	2 mg
Sodium Ascorbate	10 mg
Compressed tablet or opaque soft gel capsule	

It will be appreciated that the instant specification and claims are set forth by way of illustrati n and not limitation, and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS

1. A composition for reducing the likelihood of heart attacks, strokes or peripheral vascular diseases in patients susceptible thereto comprising an amount effective therefor of a composition comprising

i) vitamin B_6 and

ii) at least one of

a) betaine

b) choline

c) lecithin

d) vitamin B_{12} or

e) folic acid.

2. A composition according to claim 1, wherein ii) comprises betaine.

3. A composition according to claim 1, wherein ii) comprises choline.

4. A composition according to claim 1, wherein ii) comprises lecithin.

5. A composition according to claim 1, wherein ii) comprises vitamin B_{12}.

6. A composition according to claim 1, wherein ii) comprises folic acid.

7. A composition according to claim 1, wherein ii) comprises vitamin B_{12} plus folic acid.

8. A composition according to claim 1, containing about 10 to 1000 parts by weight of vitamin B_6, when present about 5 to 1000 parts by weight of betaine, choline, and lecithin, and when present about 0.03 to 1 part by weight of folic acid and 0.0005 to 1 part by weight of vitamin B_{12}.

9. A composition according to claim 1, containing about 25 to 500 parts by weight of vitamin B_6, when present about 25 to 250 parts by weight of betaine, choline, and lecithin, and when present about 0.2 to 1.0 parts by weight of folic acid and 0.0005 to 1 part by weight of vitamin B_{12}.

10. A composition according to claim 1, in the form of a tablet or capsule, an orally administrable liquid, or an injectable solution or suspension.

11. A composition according to claim 6, in the form of a tablet or capsule.

12. A method of reducing the likelihood of heart attacks, strokes or peripheral vascular diseases in a patient susceptible thereto comprising administering an amount effective therefor of vitamin B_6 plus at least one of (a) betaine, (b) choline, or (c) lecithin, (d) vitamin B_{12} or (e) folic acid.

13. The method according to claim 9, wherein there is administered to such patient betaine.

14. The method according to claim 9, wherein there is administered to such patient choline.

15. The method according to claim 9, wherein there is administered to such patient lecithin.

16. The method according to claim 9, wherein there is administered to such patient vitamin B_{12}

17. The method according to claim 9, wherein there is administered to such patient folic acid.

18. The method according to claim 9, wherein there is administered to such patient vitamin B_{12} and folic acid.

19. The method according to claim 9, wherein to th pati nt there are daily administered about 10 to 1000 mg of vitamin B_6, and at least one of (i) about 25 to 250 mg of at least one of at least one of betaine, choline, and lecithin, and (ii) about 0.2 to 0.8 mg of folic acid and/or 0.1 to 0.5 mg of vitamin B_{12}.

20. A method of reducing the concentration of homocysteine in the blood of a patient in need thereof comprising administering to such patient an amount effective therefor of vitamin B_6 plus at least one of (a) betaine, (b) choline, (c) vitamin B_{12} or d) folic acid.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13899

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/14, 31/205, 31/495, 31/68, 31/685, 31/70
US CL :514/52, 77, 78, 249, 345, 556, 642
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/52, 77, 78, 249, 345, 556, 642

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A, 347,864 (Strydom) 27 December 1989, see entire document.	1-3, 5-14, 16-20
X	US, A, 4,687,782 (Brantman et al.) 18 August 1987, see entire document.	1, 3, 5-10, and 20
X ---- Y	US, A, 4,902,718 (Bayless et al.) 20 February 1990, see entire document.	1, 2, 5-13, 16-20 ---------- 1-3, 5-14, 16-20
Y	US, A, 2,931,818 (McQuarrie et al.) 05 April 1960, column 1, lines 21-23 and lines 40-50.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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